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The mineral concretions in the midgut of Tomocerus minor (Collembola) : microprobe analysis and physioecological significance

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INTRODUCTION

The midgut of Tomocerus minor is characterized by a great number of intracellular mineral concretions. Classical histochemical methods give only incomplete results of their chemical composition. Our aim was to clarify the function of these inclusions. They should be regarded as the main site of calcium, potassium, magnesium salts deposition in the midgut cells. Microprobe analysis permits to find a lot of other mineral elements (Al, Si, P, S, Cl, Na, Fe). In the present work the emphasis is placed on the possibility for these concretions to capt foreign or toxic substances.

MATERIAL AND METHODS

Animals.

Individuals of *Tomocerus minor* Lubbok have been collected in the Rhine forests around Strasbourg. They were raised on a plaster substrate and distilled water was added to maintain a high relative humidity.

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Microprobe Analysis.

The animals were anaesthesized with ethylic ether and immediately killed by decapitation; the abdominal cavity was then opened, the midguts dissected and cleared of their contents; they were flattened on mylar slides and coated with a thin layer of evaporating carbon. All samples were analysed with an electron microprobe analyser CAMECA MS 46. The instrument was equiped with wave-length dispersive spectrometers under the following conditions: accelerating voltage: 15 KV; beam current: 50 or 100 nA; incident electron beam diameter: 1 μm. The X-Rays emitted by the preparations are dispersed by two crystal detectors: P.E.T. (Pentaerythritol) and K.A.P. (Acid potassium phtalate). The thick plastic embedded sections (1 or 2 μm) have been analysed under the same conditions; fixation procedures have been described elsewhere (HUMBERT, 1974). The thick paraffin embedded sections (5 μ) fixed with the Carnoy fixative as well as whole flattened midguts have also been analysed with a scanning electron microscope CAMEBAX equiped with an energy dispersive spectrometer TRACOR NORTHERN. Some areas of the midgut have been compared to point analyses.

Following aqueous solutions of toxic salts have been added to the food of different lots of insects: 0,5 % uranyl acetate, 1 % strontium chloride, 0,1 M lead nitrate, or 1 % manganese chloride. Ferritin has also been added to the fond; an other lot of insects was placed on iron ore. The animals were raised for 3 to 30 days on these different substrates, their midguts were then dissected and analysed as described above.

RESULTS

1. Chemical constitution of the midgut mineral concretions (Fig. 3, a, b)

The histochemical analysis completed by microprobe analysis (HUMBERT 1976 in Press) shows that the mineral inclusions are mainly built up with Ca, K, Mg, Na phosphates. Non negligible amounts of S, Cl, Al and Si are present. Traces of iron and sometimes manganese have also been detected. All these elements except aluminium and silicon have been traced in various mineral accumulating organs of a lot of Insects; they can be considered as natural constituants of intracellular mineral inclusions (BALLAN-DUFRANÇAIS 1970; BALLAN *and al.* 1971; MARTOJA 1972, 1974; LHONORÉ 1973; HUBERT 1974; MARTOJA 1974). Non common elements such as aluminium and silicon have already been detected in some Invertebrates (MARTOJA and MARTOJA, 1973) and even Vertebrates (TRUCHET and MARTOJA 1973).

The histochemical and microprobe analyses also show that we can compare the mineral composition of the concretions to the composition of the midgut contents. (HUMBERT, unpublished).

Ca and P X-Ray images of three analysed areas (Fig. 1 a, 2 a b) of the midgut of *Tomocerus minor* show a general distribution of these two main elements *. Energy dispersive analyses of the same areas show that Al and Mg are not found in each area (area 1, e.g.); elements such as Cl are more abundant in area 1 than in area 2 and 3; Ca is more abundant in area 2 and 3 than in area 1.

* With the skillfull technical assistance of Mr. P. BOUMATI.

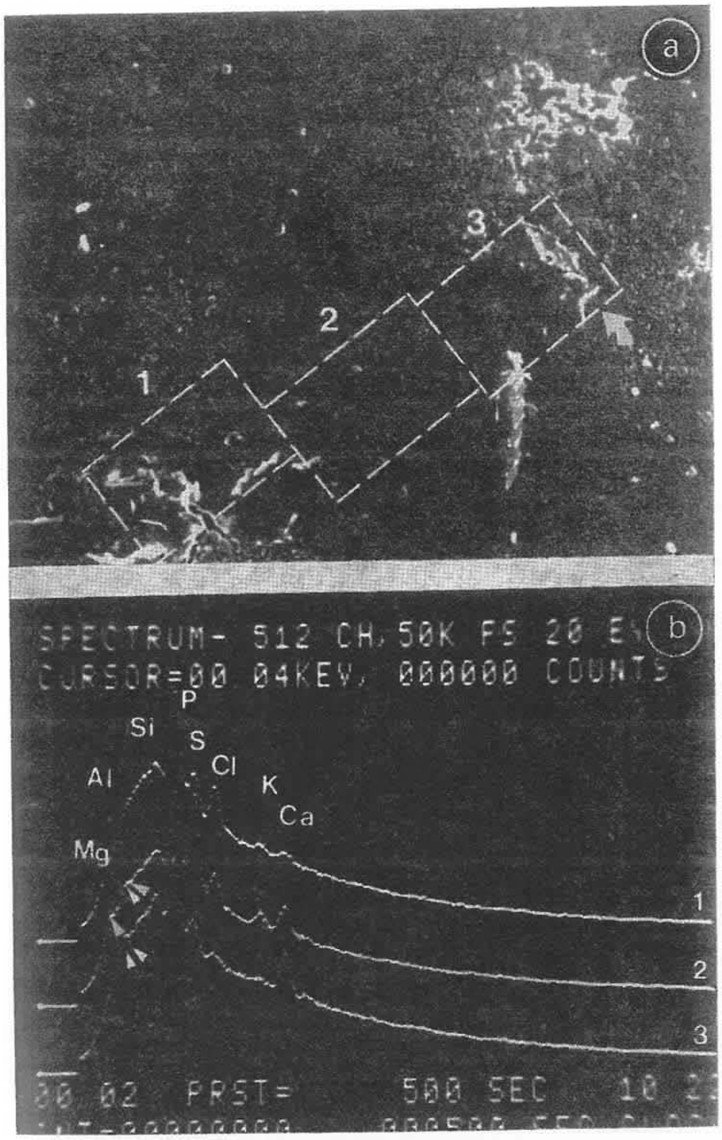

FIG. 1. — *a*. Secondary electron image of a flattened midgut of Tomocerus minor obtained with a scanning electron microscope CAMEBAX; 1, 2, 3, represent the three areas which have been analysed (× 100). ↗ anterior part of the midgut.

b. Principal energy dispersive X-Ray spectra obtained with the three analysed areas (with an energy dispersive analyser Tracor Northern). Mg : Magnesium ; Al : Aluminium ; Si : Silicon ; P : Phosphorus ; S : Sulphur ; Cl : Chlorine ; K : Potassium ; Ca : Calcium. Mg (▲) and Al (▲▲) have discrete emissions and are not found in area 1. All other elements exist in each area; Ca is more abundant in area 2 and 3, K in area 2.

This explains why each element is not always found when using a point analysing system (Cameca MS 46, fig. 3 a, b, e.g.). These two methods are complementary in allowing to deduce the mineral composition of the midgut cells.

2. **Experimental intoxication** (Fig. 3, c, 4, a-e).

The classical plaster substrate was replaced by iron ore powder or by washed sand added with different salt solutions. Food was added to the new substrate.

A lot of animals was then placed on the iron ore. After 2 or 3 days they were dissected and their midguts analysed. Among the natural mineral constituants of the midgut epithelium and especially of the mineral concretions such as Ca, K, P, Na, S, a lot of iron (Fe) has been found (Fig. 3 c). (All mineral elements have not been represented in each figure). This is to be attributed to the presence of iron on the substrate which has been capted by the midgut cells. The mortality of the animals was high (about 100 % after 5 days). It may be that the concentration of iron (and phosphorus) is too high and becomes really toxic.

In fig. 4 a, we show the results obtained with an uranium salt: uranyl acetate; a 0,5 % aqueous solution has been added to the substrate and food was added. The animals were dissected after 3 days and their midguts analysed. The principle peaks are represented and show the Mα line emission of uranium associated with Ca, K, P. The mortality of the animals was a high as with iron ore (about 100 % after 5 days).

A solution of 1 % ferritin * added to the food was found to be not toxic; the animals were all alive and healthy after one month. They were dissected, fixed for electron microscopy and semi-thin sections have been analysed. Ca, Os (coming from the fixation) and Fe have been represented in fig. 4, b. The emission of these elements is not so important as in other analyses (see fig. 3, c) because the analysis, made on semi-thin sections, concerns only non diffusible ions (the diffusible ions have been lost during fixation procedures).

The same experiments have been made with manganese chloride. They show that midgut epithelium is also able to capt Mn ions. The mortality was about 100 % after 7 days. The main emissions have been represented in fig. 4, c.

An other lot of Collembola was put on a lead salt substrate (lead acetate). With the concentration used, the mortality was not so high as before. It reached about 100 % after 30 days only. Animals in presence of the intoxicated substrate for 5 days were dissected and analysed as before. A great amount of lead can be found in the midgut epithelium beside Ca, K and P which are represented in fig. 4, d.

The last series of tests were done with strontium chloride. The methods used were the same as before. An important emission of strontium is shown in fig. 4, e beside Ca and K. This element too is capted by the midgut cells. The mortality of the animals proved also high (about 100 % in 5 days).

* (A protein rich in iron).

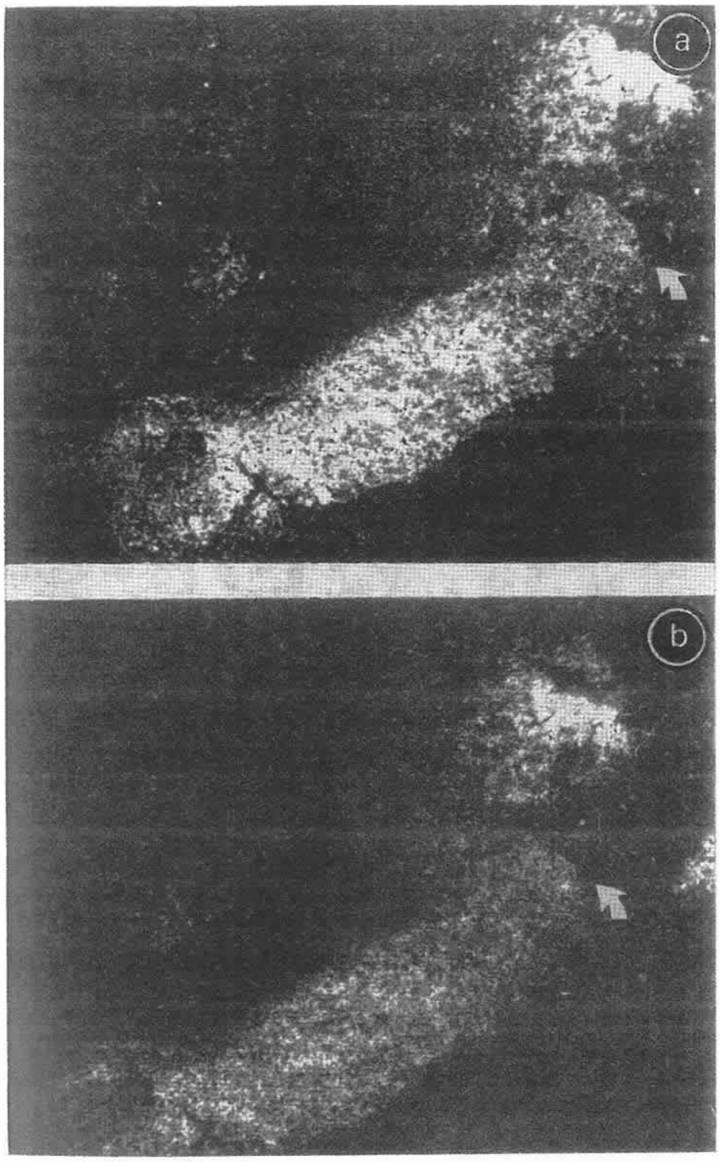

FIG. 2. — *a* and *b* represent X-Ray images of Calcium (a) and Potassium (b) of the sample represented in fig. 1 a. The images reveal a general distribution of Calcium and Potassium. Each white spot represents an emission of Calcium (Ca) or Potassium (b). Outside the midgut the points represent background emissions. ↗ Anterior part of the midgut.

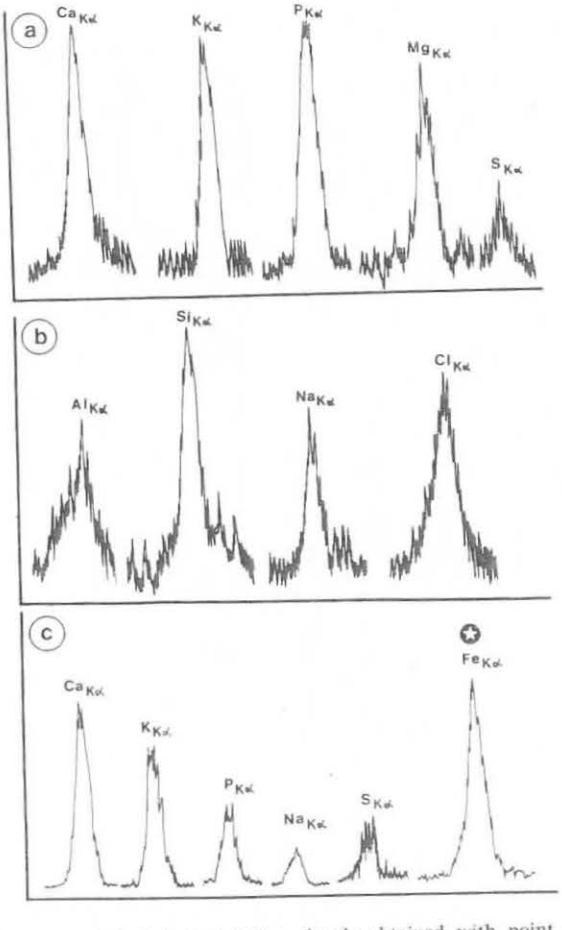

FIG. 3. — *a* and *b*. Principal X-Ray signals obtained with point analyses of flattened, dried and coated midgut epithelium (with a wave length dispersive analyser Cameca MS 46). Some elements such as Mg, Al, Na have weak emissions and could not be found easily with the energy dispersive analyser. Number of X-Ray counts per second of the principal emission rays:
Ca ($K\alpha$: 700 ; K ($K\alpha$: 500) ; P ($K\alpha$: 600) ; Mg ($K\alpha$: 130) ; S ($K\alpha$: 150) ; Al ($K\alpha$: 90) ; Si ($K\alpha$: 200) ; Na ($K\alpha$: 40) ; Cl ($K\alpha$: 60).

c. Principal emissions of the point analyses of flattened, dried and coated midgut epithelium (Iron ore was added to the substrate). Note the important emission of iron (Fe).
Principal rays :
Ca ($K\alpha$: 500) ; K ($K\alpha$: 120) ; P ($K\alpha$: 220) ; Na ($K\alpha$: 130) ; S ($K\alpha$: 20) ; Fe ($K\alpha$: 550).

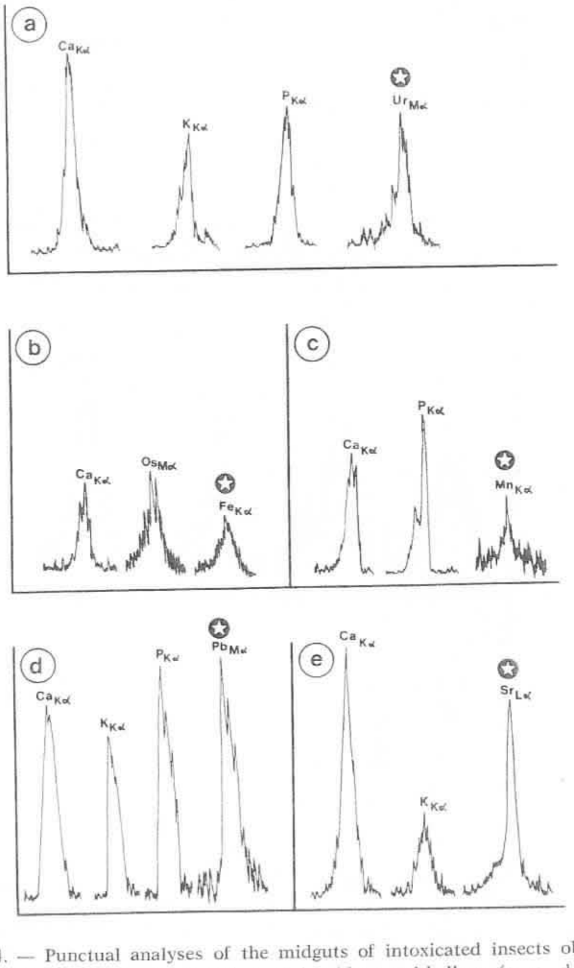

FIG. 4. — Punctual analyses of the midguts of intoxicated insects obtained with flattened, dried and coated midgut epithelium (a, c, d, e) or with fixed semi-thin sections (Glutaraldehyde - Osmium (b).
The introduced element is represented by the following sign ☆
Principal X-Ray emissions of the main elements (in counts per sec.) of the intoxicated insects.

a) with uranyl acetate : Ca ($K\alpha$: 560) ; K ($K\alpha$: 300) ; P ($K\alpha$: 340) ; Ur ($M\alpha$: 320).
b) with ferritin : Ca ($K\alpha$: 200 ; Os ($M\alpha$: 80) ; Fe ($K\alpha$: 120).
c) with manganese chloride : Ca ($K\alpha$: 600) ; P ($K\alpha$: 400) ; Mn ($K\alpha$: 60).
d) with lead nitrate : Ca ($K\alpha$: 900) ; K ($K\alpha$: 450) ; P ($K\alpha$: 800) ; Pb ($M\alpha$: 800) (Lead).
e) with strontium chloride : Ca ($K\alpha$: 800) ; K ($K\alpha$: 240) ; Sr ($L\alpha$: 520).

DISCUSSION AND CONCLUSION

It is clear that the intracellular mineral elements (natural or introduced by intoxication) are coming from the alimentary bowlfull and are accumulated in the midgut cells. The natural composition of the mineral concretions may be compared with the mineral composition of the midgut contents (HUMBERT, unpublished).

CHRISTIANSEN (1970) showed that Collembola feed with substrate clay if no food is added. So, the ingestion of substrate is a natural behaviour of Collembola. It even enables the insects to insure the growth of their juvenile phase (BARRA, 1976).

The presence of Al and Si in the midgut is to be attributed to the fact that these elements are normal constituants of the plaster (Alumine silicates). These elements, rather unusual in biological tissues (MARTOJA and MARTOJA, 1973; TRUCHET and MARTOJA, 1973) seem to have been « capted » by the mineral intracellular concretions (HUMBERT, 1977).

Recently, it has been shown that mineral inclusions are temporary accumulations which are periodically expelled by apocrine or holocrine extrusion and especially among Collembola by the renewal of the whole midgut epithelium (HUMBERT, 1974).

JEANTET *and al.* (1974) have shown that ant workers are able to resist to various intoxications (baryum, copper, lead nitrates, cobalt acetate, cadmium chloride). This resistance could be due to the presence of an epithelium rich in mineral inclusions. The importance of the midgut in the ionic retention has been demonstrated by BOWEN (1950). *Manganese added to the food has been found in the midgut cells of Social Vespidae.

The intestinal epithelium may be considered as an ionic barrier, not only for the classical cations brought by the food but also for the heavy elements. This barrier can be more or less efficient according to the nature of the ingested elements or to their concentration. This seems to be the case with the Collembola which have been analysed. If the concentration of the toxical element is too high in the midgut content, it can not be completely capted by the midgut concretions; therefore, a great proportion gets into the hemolymph and causes rapid mortality (with uranyl acetate, strontium chloride, manganese chloride and iron ore).

With a low concentration of ferritin (which proved less toxic than iron ore), the animals were able to capt regularly the element and excrete it by the way of midgut epithelium renewal. So an element such as iron, which is important in biology, is able to be capted by the midgut concretions and eliminated regularly. If the same element is in a too high concentration such as iron ore, this biological element gets toxic.

The intoxication with a heavy toxic element such as lead nitrate is easily supported for a longer period. Because the animals are able to moult, their midgut epithelium after capting toxic ions is replaced by a new one. JEANTET thinks with reason that the renewal of mineral concretions interferes with the resistance capacity of an insect. This preliminary study shows that the

midgut cells of Collembola are able to precipitate and to capt foreign and toxic ions. This is to be attributed to the presence of intracellular mineral concretions which give the animal the capacity to resist to chemical agressions. But these elements must be in low concentrations. Other experiments are on the way to show if other cellular components participate in the elimination of toxic elements. This resistance capacity plays a great part in the ecology of these insects which have a very large geographic distribution all over the world.

It appears thus that Collembola, as many other Insects belonging to different orders (Homoptera, Tricoptera, Coleoptera) are « equiped » to face mineral pollutions.

ABSTRACT

The midgut of Tomocerus minor is characterized by the presence of a great number of intracellular mineral concretions. Histochemical methods and microprobe analysis have enabled us to study the chemical composition of these inclusions. They are mainly built up with calcium, potassium, magnesium, sodium phosphates. Sulphur, aluminium, silicon, chlorine, traces of iron and sometimes of manganese are also present. These mineral elements associated with an organic matrix constituted by polysaccharides and probably proteins are amorphous. Aluminium and silicon which are impossible to detect by classical histochemical methods are easily detected by the microanalyser. These elements come from the plaster substrate. To define precisely the physiological significance of the mineral concretions, individuals have been isolated on iron ore, or on ferritin added food. Iron was found in the midgut probably associated to the spherical concretions. Toxic salts (uranium, lead, strontium, manganese) were added to the food and were also found incorporated to the midgut epithelium and probably « capted » by the concretions. Other cellular components may well be involved in the excretion of these substances.

Collembola seem to have a high resistance capacity towards toxical elements and this is probably due to the excretory part played by the intracellular mineral concretions and by the whole midgut epithelium.

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